PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 12 April 2000 (12.04.00)	
International application No. PCT/US99/16242	**Applicant's or agent's file reference** EMU143PCT
International filing date (day/month/year) 16 July 1999 (16.07.99)	**Priority date** (day/month/year) 17 July 1998 (17.07.98)
Applicant SHAFER, David, A.	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

17 February 2000 (17.02.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Pascal Piriou
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BG

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12Q 1/68, C12P 19/34, C07H 21/02, 21/04

A1

(11) International Publication Number: **WO 00/04192**

(43) International Publication Date: 27 January 2000 (27.01.00)

(21) International Application Number: PCT/US99/16242

(22) International Filing Date: 16 July 1999 (16.07.99)

(30) Priority Data:
60/093,219 17 July 1998 (17.07.98) US

17 Jan 01/30 mwz

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(81) Designated States: AU, CA, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
With amended claims.

(54) Title: METHODS FOR DETECTING AND MAPPING GENES, MUTATIONS AND VARIANT POLYNUCLEOTIDE SEQUENCES

(57) Abstract

The present invention includes a number of related designs for gene probe components, multilinking components and signaling components, all of which are modular in nature and can be used together or in part. These components are generally joined together in composite structures by hybridization of complementary sub-segments, called linkers. The reporters of the present invention are also designed to be conjoined into arrays that can provide amplified signaling. The multilinking components of the present invention may be interposed between the probe and the reporter units and provide for the binding of multiple reporters. These probe and signaling methods also include means to achieve mixed-color labeling that is specific to each target. The present invention is useful for detecting target sequences in a wide variety of formats including, but not limited to, membrane formats, in situ formats, and on various solid substrate chip formats.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/16242

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 92/17484 A1 (RESEARCH CORPORATION TECHNOLOGIES, INC.) 15 October 1992, see entire document.	1-57
Y	NILSSON et al. Padlock probes: circularizing oligonucleotides for localized DNA detection. Science. 30 September 1994, Vol. 265, pages 2085-2088, see entire document.	1-57
Y	Database CAPLUS on STN, VOGELBACKER et al. DNA dendrimers: assembly and signal amplification. Book of Abstracts, 213th ACS National Meeting, San Francisco. April 1997, abstract only.	1-57

US 09/744097

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PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference EMU143PCT	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US99/16242	International filing date *(day/month/year)* 16 JULY 1999	Priority date *(day/month/year)* 17 JULY 1998
International Patent Classification (IPC) or national classification and IPC Please See Supplemental Sheet.		
Applicant EMORY UNIVERSITY		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets.

   [X] This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority. (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 12 sheets.

3. This report contains indications relating to the following items:

   I [X] Basis of the report

   II [ ] Priority

   III [ ] Non-establishment of report with regard to novelty, inventive step or industrial applicability

   IV [ ] Lack of unity of invention

   V [X] Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

   VI [ ] Certain documents cited

   VII [ ] Certain defects in the international application

   VIII [ ] Certain observations on the international application

Date of submission of the demand	Date of completion of this report
17 FEBRUARY 2000	21 NOVEMBER 2000
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer Dorothea Laurence for JEFFREY FREDMAN
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

Form PCT/IPEA/409 (cover sheet) (July 1998)*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US99/16242

I. Basis of the report

1. With regard to the **elements** of the international application:*

[X] the international application as originally filed

[X] the description:
pages (See Attached) , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

[X] the claims:
pages (See Attached) , as originally filed
pages _____ , as amended (together with any statement) under Article 19
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

[X] the drawings:
pages (See Attached) , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

[X] the sequence listing part of the description:
pages (See Attached) , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

[ ] the language of publication of the international application (under Rule 48.3(b)).

[ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. ~~With regard to any nucleotide and/or amino acid sequence~~ disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

[X] contained in the international application in printed form.

[X] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] The statement that the information recorded in computer readable form is identical to the writen sequence listing has been furnished.

4. [X] The amendments have resulted in the cancellation of:

[X] the description, pages NONE

[X] the claims, Nos. NONE

[X] the drawings, sheets/~~fig~~ NONE

5. [ ] This report has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

** Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

*** Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US99/16242

V. Reasoned statement under Article 35(2) with regard t novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. statement

Novelty (N)	Claims	9, 10, 13-55	YES
	Claims	1-8, 11, 12	NO
Inventive Step (IS)	Claims	NONE	YES
	Claims	1-55	NO
Industrial Applicability (IA)	Claims	1-55	YES
	Claims	NONE	NO

2. citations and explanations (Rule 70.7)

Claims 1-8, 11, 12 lack novelty under PCT Article 33(2) as being anticipated by Urdea et al (U.S. Patent 5,681,697).

Urdea teaches reporters which comprise branched, labeled double stranded polynucleotide sequences having multiple linkers with terminator DNA sequences where in the linkers are complementary to a second linker sequence, but will not hybridize to a target sequence (figure 12, see Amp1 and Amp2). With regard to the claim for linkers which are not complementary to one another, see figure 12, LE1 and LE2.

Claims 9, 10, 13-55 lack an inventive step under PCT Article 33(3) as being obvious over Nilsson et al (Science (1994) 265:2085-2088) in view of Urdea et al (U.S. Patent 5,681,697).

Nilsson teaches a method for detecting a target nucleic acid comprising the steps: a) rendering the target nucleic acid single stranded (page 2087, figure 3 legend), b) hybridizing a padlock probe, which comprises two separate probe ends which hybridize at tandem locations on the target nucleotide sequence and which are joined by a linker which does not hybridize to the target sequence (page 2087, figure 3 legend), c) ligating the ends to form a closed circle (page 2087, figure 3 legend), e) denaturing to remove unligated probe (page 2087. figure 3 legend), f) detecting the presence or absence of the probe (page 2087, figure 3).

Nilsson does not teach the use of multiple linkers or of dendrimers, nor does Nilsson teach the use of simultaneous sense and antisense detection, nor does Nilsson teach capture of the probes to a solid support. Nilsson also does not teach the use of separate label reporters or terminated units of the linkers nor the use of multipe different labels.

Urdea teaches reporters which comprise branched. labeled double stranded polynucleotide sequences having multiple linkers with terminator DNA sequences where in the linkers are (Continued on Supplemental Sheet.)

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

CLASSIFICATION:
The International Patent Classification (IPC) and/or the National classification are as listed below:
IPC(7): C12Q 1/68; C12P 19/34; C07H 21/02, 21/04 and US Cl.: 436/6, 91.1, 91.2; 536/22.1 24.3 24.31 24.32, 24.33

I. BASIS OF REPORT:

This report has been drawn on the basis of the description,
page(s) 1-74, as originally filed.
page(s) NONE, filed with the demand.
and additional amendments:
NONE

This report has been drawn on the basis of the claims,
page(s) 77-79, 81, 82, as originally filed.
page(s) 75, 76, 80, 83-91 as amended under Article 19.
page(s) NONE, filed with the demand.
and additional amendments:
NONE

This report has been drawn on the basis of the drawings,
page(s) 1-22, as originally filed.
page(s) NONE, filed with the demand.
and additional amendments:
NONE

This report has been drawn on the basis of the sequence listing part of the description:
page(s) 1-34, as originally filed.
pages(s) NONE, filed with the demand.
and additional amendments:
NONE

V. 2. ~~REASONED STATEMENTS - CITATIONS AND EXPLANATIONS~~ (Continued):
complementary to a second linker sequence, but will not hybridize to a target sequence (figure 12, see Amp1 and Amp2). With regard to the claim for linkers which are not complementary to one another, see figure 12, LE1 and LE2. Urdea teaches capture of the reporters onto a solid support (figure 12). Urdea teaches the use of multiple separate label reporters and terminated units (figure 12). Urdea also teaches the use of two distinct labels (column 16, lines 15-20 and figure 13).

It would have been prima facie obvious to an ordinary practitioner at the time the invention was made to combine the multimers of Urdea in the padlock probe method of Nilsson since Urdea states "The invention increases both the sensitivity and specificity of such assays, by reducing the incidence of signal generation that occurs in the absence of target and does not involve a substantial increase in either time or cost relative to current assay configurations (column 2, lines 45-49)". An ordinary practitioner would have been further motivated to detect both the sense and antisense strands in situations where the increased sensitivity was necessary for successful detection since an ordinary practitioner would recognize that either strand or both strands of target are detectable by hybridization assays.

---------- NEW CITATIONS ----------
NONE

AMENDED CLAIMS

[received by the International Bureau on 16 December 1999 (16.12.99);
original claim 5, 7, 18, 32, 36, 38-40, 43, 48, 55 and 57 amended;
remaining claims unchanged (12 pages)]

1. A GENE-TAG reporter, suitable for joining to a probe either alone or in combination with another GENE-TAG reporter, comprising a labeled, double-stranded polynucleotide sequence having one or more first linkers, said first linker comprising a single-stranded nucleotide sequence hybridizable to a complementary single-stranded nucleotide sequence, wherein said first linkers is not hybridizable to a target sequence of said probe.

2. The GENE-TAG of Claim 1, wherein said GENE-TAG further comprises one or more second linkers opposed to said first linker, said second linker comprising a single-stranded nucleotide sequence hybridizable to a complementary nucleotide sequence, wherein said second linker is not hybridizable to a target sequence of said probe.

3. The GENE-TAG of Claim 2, wherein said first and second linkers are complementary.

4. A reporter array, suitable for joining to a probe, comprising two or more of the GENE-TAGs of Claim 3, linked together end-to-end by hybridization of the second linker of a first GENE-TAG to the first linker of a second GENE-TAG, and optionally, additional GENE-TAGs linked to the remainder of the array by hybridization of the first linker of each subsequent GENE-TAG to the second linker of the preceding GENE-TAG in the array, to form a chained or branched configuration having one or more terminal ends, said terminal end comprising a second linker of a terminal GENE-TAG.

5. The reporter array of Claim 4, further comprising one or more terminators, wherein said terminator comprises a single-stranded nucleotide sequence complementary to the second linker of one or more terminal GENE-TAGs, whereby said terminator forms said terminal end.

6. The GENE-TAG of Claim 2, wherein the first and second reporter linkers are not complementary.

7. A reporter array, suitable for joining to a probe, said reporter array comprising two or more of first GENE-TAGs of Claim 6, linked indirectly by one or more second GENE-TAGs of Claim 6 by end-to-end hybridization of the second linker of a first GENE-TAG to the first linker of a second GENE-TAG, and hybridization of the second linker of a second GENE-TAG to the first linker of said first GENE-TAG, and optionally, additional non-complementary GENE-TAGs linked to the remainder of the array by hybridization of the first linker of each subsequent GENE-TAG to the complementary second linker of the preceding GENE-TAG in the array, to form a chained or branched configuration.

8. A probe, suitable for hybridizing to a target nucleotide sequence, said probe comprising a first terminal linker, a second terminal linker, and a central sequence complementary to said target nucleotide sequence, wherein said first and second terminal linkers comprise single-stranded nucleotide sequences hybridizable to a complementary nucleotide sequence, but not hybridizable to the target nucleotide sequence of said probe.

9. A composite probe, suitable for hybridizing to a target nucleotide sequence, comprising at least one probe, said probe comprising a polynucleotide sequence complementary to said target nucleotide sequence, said composite probe further comprising a polynucleotide ring unit, wherein said polynucleotide ring unit is joined to said probe to form an incipient closed loop.

10. The composite probe of Claim 9, comprising a first and second probe, wherein said first probe comprises a sequence complementary to a first region of said target nucleotide sequence, and wherein said second probe comprises a sequence complementary to a second region of said target sequence, wherein said ring unit is joined to said first probe and to said second probe, thereby forming an incipient closed loop.

reporters, wherein said second reporter produces a signal distinct from a signal produced by said first reporter;

(f) treating the probes and RING-LOCK Units, at this step or another step, to effect cross linking or to increase binding;

(g) hybridizing said first and second sense probes to the sense strand, thereby forming a sense hybridized complex; and hybridizing said first and second antisense probes to the antisense strand, thereby forming an antisense hybridized complex;

(h) ligating said first and second sense probes, thereby forming a closed loop or an incipient closed loop around the sense target strand; and ligating said first and second antisense probes, thereby forming a closed loop or an incipient closed loop around the antisense target strand;

(i) joining at least one first single-stranded polynucleotide to said first distal linker of said first sense probe, if not previously joined; joining at least one first single-stranded polynucleotide to said second distal linker of said second sense probe, if not previously joined; joining at least one second single stranded polynucleotide to said first distal linker of said first antisense probe, if not previously joined; joining at least one second polynucleotide to said second distal linker of said second antisense probe, if not previously joined;

(j) joining one or more first reporters to at least one said first single-stranded polynucleotide, if not so previously joined; joining one or more second reporters to at least one said second single-stranded polynucleotide, wherein said second reporter produces a signal distinct from said first reporter;

(k) determining whether the sense target sequence is present by detecting the presence or absence of said first reporter or reporters; or determining whether the antisense target sequence is present by detecting the presence or absence of said second reporter or reporters; or both.

19. The method of Claim 14, for use under liquid or near-liquid hybridization conditions, wherein said first probe comprises a distal end that is joined to a capture moiety, and said

stranded target nucleotide sequence and a nucleic acid probe, said WRAP-PROBE comprising a central sequence complementary to the single-stranded target nucleotide sequences, and further comprising a probe linker at one or both terminal ends, said probe linker comprising a single-stranded nucleotide sequence that does not hybridize to the target sequence; wherein said probe linker sequence is joined to one or more reporters, either prior to or subsequent to the hybridization of the probe to the target sequence;

c) washing to remove any unbound probe;

d) joining said reporter to said probe linker, if not previously joined;

e) detecting the presence or said reporter or reporters to indicate the target sequence.

29. The method of Claim 28, wherein the probe comprises a first terminal probe linker.

30. The method of Claim 28, wherein the probe comprises a first terminal probe linker and a second terminal probe linker.

31. The method of Claim 30, wherein the reporter is a labeled, double-stranded polynucleotide sequence, known as a GENE-TAG, having one or more said first reporter linkers, said first reporter linker comprising a single-stranded nucleotide sequence hybridized to the first terminal probe linker.

32. The method of Claim 30, wherein one or more reporters comprise a reporter array, said array comprising a first labeled, double-stranded polynucleotide sequence, known as a GENE-TAG, linked together end-to-end by hybridization to one or more GENE-TAGS, wherein said first GENE-TAG comprises one or more first reporter linkers, said first reporter linker comprising a single-stranded nucleotide sequence hybridizable to said first terminal probe linker, and one or more second reporter linkers hybridized to one or more first reporter linkers of a second GENE-TAG, and wherein said second GENE-TAG further comprises one or more second reporter linkers, and optionally, additional GENE-TAGs linked to the remainder of the array by hybridization of the first reporter linker of each subsequent GENE-

TAG to the second reporter linker of the preceding GENE-TAG in the array, to form a chained or branch configuration having one or more ends, wherein said terminal end comprises the second reporter linker of a terminal GENE-TAG.

33. The method of Claim 32, further comprising one or more terminators, said terminator comprising a single-stranded polynucleotide sequence complementary to said second reporter linker of one or more terminal GENE-TAGs, such that said terminator forms said terminal end.

34. The method of Claim 28, wherein the reporter is joined directly to the first terminal probe linker.

35. The method of Claim 28, wherein the reporter is joined indirectly to the first terminal probe linker, further comprising a multi-linking unit interposed between said reporter and terminal probe linker, said unit known as a Multi-LINKER, comprising a first polynucleotide comprising a first terminal unit linker, a second terminal unit linker, and at least one internal linker, wherein said first terminal unit linker is hybridized to said first terminal probe linker, and wherein at least two of said internal and second terminal unit linkers are hybridized to one or more reporters, and wherein said first and second terminal unit linkers and said internal linker are not hybridizable to a target sequence of said probe.

36. The method Claim 35, further comprising one or more additional polynucleotides, said additional polynucleotide comprising a first terminal unit linker, a second terminal unit linker, and at least one internal linker, wherein the first polynucleotide is joined to the additional polynucleotide by hybridization of the additional polynucleotide to the internal or second terminal linker of the first polynucleotide, and wherein said first and second terminal linkers and said internal linker are not hybridizable to a target sequence.

37. The method of Claim 30, wherein the reporter is a short oligonucleotide having a label unit.

38. A WRAP-LOCK method for providing circular enclosure of the target polynucleotide strand with a WRAP-PROBE, comprising:

a) providing the WRAP-PROBE of Claim 30;

b) providing a RING-LOCK Unit comprising at least two single-stranded polynucleotides, wherein at least one single-stranded polynucleotide is joined or suitable for joining to said first terminal probe linker, and wherein at least one single-stranded polynucleotide is joined or suitable for joining to one or more reporters;

c) treating the probe and RING-TAIL unit, at this step or another step, to effect cross linking or to increase binding;

c) hybridizing the WRAP-PROBE to the target strand, thereby forming a hybridized complex;

d) joining at least one single-stranded polynucleotide to said first terminal probe linker, if not previously joined,

e) providing a looping polynucleotide comprising a first region complementary to said second terminal probe linker, and a second region complementary to said RING-TAIL Unit, wherein said looping polynucleotide hybridizes to the second terminal probe linker and the RING-TAIL Unit, thereby forming a closed loop about the target strand;

f) joining a reporter or reporters to at least one ring-tail linker, if not previously attached; and

g) detecting the presence of reporter to indicate the target sequence.

39. A DOUBLE WRAP-LOCK method of simultaneously detecting a target sequence on both a sense and anti-sense strand of DNA, comprising:

a) providing a double-stranded DNA comprising an antisense strand and a sense strand, wherein both said antisense strand and said sense strand comprise a target sequence;

b) providing a sense WRAP-PROBE of Claim 30, said sense WRAP-PROBE probe having a central sequence complementary to the sense strand target sequence;

c) providing an antisense WRAP-PROBE probe of Claim 30, said antisense WRAP-LOCK probe having a central sequence complementary to the antisense strand target sequence;

d) providing a first RING-TAIL Unit comprising at least two first single-stranded polynucleotides, wherein at least one first single-stranded polynucleotide is joined or suitable for joining to said first terminal probe linker of said sense WRAP-PROBE, and wherein at least one first single-stranded polynucleotide is joined or suitable for joining to one or more first reporters;

e) providing a second RING-LOCK Unit comprising at least two second single-stranded polynucleotides, wherein at least one second single-stranded polynucleotide is joined or suitable for joining to said first terminal probe linker of said antisense WRAP-PROBE, and wherein at least one second single-stranded polynucleotide is joined or suitable for joining to one or more second reporters, wherein said second reporter produces a signal distinct from said first reporter;

(f) treating the WRAP-PROBEs and the RING-LOCK Units, at this step or another step, to effect cross linking or to increase binding;

(g) hybridizing said sense WRAP-PROBE to the sense strand, thereby forming a sense hybridized complex; and hybridizing said antisense WRAP-PROBE to the antisense strand, thereby forming an antisense hybridized complex;

(h) joining at least one first single-stranded polynucleotide to said first terminal probe linker of said sense WRAP-PROBE, if not so previously joined; and joining at least one second single-stranded polynucleotide to said first terminal probe linker of said antisense WRAP-PROBE, if not so previously joined;

(i) providing a first looping polynucleotide comprising a first region complementary to said second terminal probe linker of said sense WRAP-PROBE, and a second region complementary to said first RING-TAIL Unit, wherein said looping polynucleotide hybridizes and thereby forms a closed loop about the target sense strand; and providing a second looping polynucleotide comprising a first region complementary to said second terminal probe linker of said antisense WRAP-PROBE, and a second region complementary to said second RING-TAIL Unit, wherein said looping

polynucleotide hybridizes and thereby forms a closed loop about the target antisense strand;

(j) joining one or more first reporters to at least one said first single-stranded polynucleotide, if not so previously joined; joining one or more second reporters to at least one said second single-stranded polynucleotide, if not previously joined, wherein said second reporter produces a signal distinct from said first reporter;

(k) determining whether the sense target sequence is present by detecting the presence or absence of said first reporter or reporters; or determining whether the antisense target sequence is present by detecting the presence or absence of said second reporter or reporters, or both.

40. A GENE-TAG method for exponential signal amplification for the detection of a target nucleotide sequence, comprising:

a) providing a single-stranded target nucleic acid sequence;

b) providing a probe comprising a sequence hybridizable to said target nucleic acid sequence, and a terminal linker sequence on one or both ends of the probe, wherein said linker sequence is a single-stranded polynucleotide hybridizable to a complementary sequence;

c) providing one or more GENE-TAGs, said GENE-TAG comprising a labeled, double-stranded polynucleotide reporter having one or more first reporter linkers and one or more second reporter linkers opposed to said first reporter linker, said reporter linkers comprising single-stranded polynucleotide sequences not hybridizable to the target sequence, wherein at least one said GENE-TAG has a first reporter linker complementary to said probe terminal linker, and wherein one or more said reporter linkers link together end-to-end by hybridization of the first reporter linker of each subsequent GENE-TAG to the second reporter linker of each preceeding GENE-TAG in the array, to form a chained or branched configuration comprising one or more terminal end, wherein said terminal end comprises the second reporter linker of a terminal

GENE-TAG; wherein said GENE-TAG is joined to said probe in this or a subsequent step;

d) hybridizing said probe to said target nucleotide sequence;

e) joining said GENE-TAG or GENE-TAGs to said probe, if not so previously joined;

f) determining the presence of the target sequence by detecting the presence of the GENE-TAG or GENE-TAGs. .

41. The method of Claim 40, wherein the first and second reporter linkers of the GENE-TAGs are complementary, such that only GENE-TAGs of one type are required to form chained or branched reporter arrays.

42. The method of Claim 41, further comprising one or more terminators, said terminator comprising a single-stranded polynucleotide sequence complementary to said second reporter linker of said terminal GENE-TAG, such that said terminator forms said terminal end.

43. The method of Claim 42, wherein the length of said chained or branched reporter arrays, and thus probe amplification, is controlled by the ratio of GENE-TAGs to said terminators.

44. The method of Claim 41, further comprising a Terminator TAG, said Terminator TAG comprising a GENE-TAG having no second reporter linker, wherein said first reporter linker of said Terminator TAG hybridizes to the second reporter linker of a terminal GENE-TAG of the chain or branch.

45. The method of Claim 44, wherein the length of said chained or branched reporter arrays, and thus probe amplification, is controlled by the ratio of GENE-TAGs to Terminator TAG.

46. The method of Claim 40, wherein only one GENE-TAG is provided, said GENE-TAG having no second reporter linker.

47. The method of Claim 40, wherein said first and second linkers of said GENE-TAGs are not complementary, such that at least two types of GENE-TAGs are required to form chained or branched arrays, wherein GENE-TAGs of a first type comprise a first reporter linker complementary to the probe terminal linker, and a second reporter linker complementary to the first reporter linker of GENE-TAG of the second type, and wherein said GENE-TAG of the second type has a first reporter linker complementary to the second reporter linker of a GENE-TAG of the first type, such that only a GENE-TAG of the first type will bind to a GENE-TAG of the second type, and only a GENE-TAG of the second type will bind to a GENE-TAG of the first type; wherein said chained or branched arrays are formed by providing GENE-TAGs sequentially, alternating between the first and second type, and thereby forming alternating layers of GENE-TAGs of a first or second type upon the probe.

48. The method of Claim 40, wherein said GENE-TAGS are constructed by PCR amplification of arbitrary template DNA using one or more modified oligonucleotides as primers, wherein each modified oligonucleotide has one or more internal spacers, whereby the first and second terminal single-stranded linkers are preserved.

49. The method of Claim 40, wherein said GENE-TAGs are constructed by hybridization of two polynucleotides, said first polynucleotide having a second terminal end complementary to the first terminal end of a second polynucleotide.

50. The method of Claim 49, wherein said first polynucleotide has an internal sequence complementary to the internal sequence of a second polynucleotide, forming a GENE-TAG having side arms, said side arms comprising the second terminal end of the first polynucleotide, and the first terminal end of the second polynucleotide, said GENE-TAGs known as TINKER-TAGS.

51. The method of Claim 40, wherein the GENE-TAG is labeled directly.

52. The method of Claim 40, wherein the GENE-TAG is labeled indirectly.

53. The method of Claim 50, wherein said TINKER-TAGs are labeled indirectly by hybridization of said side arms to short oligonucleotides conjugated to one or more labeling agents.

54. The method of Claim 53, wherein said TINKER-TAGs are labeled indirectly by hybridization of said side arms to short oligonucleotides conjugated to gold particles, said gold particles coated in silver to form dense clusters.

55. A method of constructing probes detectable on the basis of a unique signal, comprising:

a) providing a probe hybridizable to a single-stranded nucleotide sequence, wherein said probe comprises a terminal linker at one or both ends, said terminal linker comprising a single-stranded polynucleotide capable of binding to a complementary sequence;

b) preparing one or more COLOR-TAGs, said COLOR-TAG comprising a double-stranded polynucleotide reporter having a first reporter linker and a second reporter linker opposed to said first reporter linker, wherein said COLOR-TAG is labeled with one or more labels or colors, or both, and wherein said first and second reporter linkers are specific to the labels or colors employed; wherein at least one COLOR-TAG terminal linker is hybridizable to the terminal linker of said probe;

c) hybridizing one or more of said COLOR-TAGs to the probe, at this step or a later step;

e) hybridizing said probe to said target sequence;

f) hybridizing said COLOR-TAG to the probe, if not so previously hybridized;

g) determining the presence of said target sequence by detecting the presence of said COLOR-TAG.

56. The method of Claim 55, wherein said Color-TAG is labeled with two or more different labels or colors.

57. The method of Claim 55, further comprising a multilinking unit suitable for joining said probe to one or more COLOR-TAGs, said multilinking unit comprising a proximal linker for joining said multilinking unit to said probe terminal linker, and one or more distal linkers known as COLOR-LINKERS, said COLOR-LINKERS complementary to one or more of said COLOR-TAG-terminal-linkers, wherein a specific mix of COLOR-TAGS is captured by said multilinking unit depending on the type and specific ratio of COLOR-LINKERS used to prepare the multilinking unit, wherein said multilinking unit is hybridized to said COLOR-TAGS, thereby forming a COLOR-TAG complex, either prior or subsequent to the hybridization of the multilinking unit to the probe, and wherein said multilinking unit is hybridized to said probe either prior to or subsequent to the hybridization of the probe to said target sequence.

WHAT IS CLAIMED IS:

1. A GENE-TAG reporter, suitable for joining to a probe either alone or in combination with another GENE-TAG reporter, comprising a labeled, double-stranded polynucleotide sequence having one or more first linkers, said first linker comprising a single-stranded nucleotide sequence hybridizable to a complementary single-stranded nucleotide sequence, wherein said first linkers is not hybridizable to a target sequence of said probe.

2. The GENE-TAG of Claim 1, wherein said GENE-TAG further comprises one or more second linkers opposed to said first linker, said second linker comprising a single-stranded nucleotide sequence hybridizable to a complementary nucleotide sequence, wherein said second linker is not hybridizable to a target sequence of said probe.

3. The GENE-TAG of Claim 2, wherein said first and second linkers are complementary.

4. A reporter array, suitable for joining to a probe, comprising two or more of the GENE-TAGs of Claim 3, linked together end-to-end by hybridization of the second linker of a first GENE-TAG to the first linker of a second GENE-TAG, and optionally, additional GENE-TAGs linked to the remainder of the array by hybridization of the first linker of each subsequent GENE-TAG to the second linker of the preceding GENE-TAG in the array, to form a chained or branched configuration having one or more terminal ends, said terminal end comprising a second linker of a terminal GENE-TAG.

5. The reporter array of Claim 4, further comprising a one or more terminators, wherein said terminator comprises a single-stranded nucleotide sequence complementary to the second linker of one or more terminal GENE-TAGs, whereby said terminator forms said terminal end.

6. The GENE-TAG of Claim 2, wherein the first and second reporter linkers are not complementary.

7. A reporter array, suitable for joining to a probe, said reporter array comprising two or more of first GENE-TAGs of Claim 6, linked indirectly by one or more second GENE-TAGs of Claim 6 by end-to end by hybridization of the second linker of a first GENE-TAG to the first linker of a second GENE-TAG, and hybridization of the second linker of a second GENE-TAG to the first linker of said first GENE-TAG, and optionally, additional non-complementary GENE-TAGs linked to the remainder of the array by hybridization of the first linker of each subsequent GENE-TAG to the complementary second linker of the preceding GENE-TAG in the array, to form a chained or branched configuration.

8. A probe, suitable for hybridizing to a target nucleotide sequence, said probe comprising a first terminal linker, a second terminal linker, and a central sequence complementary to said target nucleotide sequence, wherein said first and second terminal linkers comprise single-stranded nucleotide sequences hybridizable to a complementary nucleotide sequence, but not hybridizable to the target nucleotide sequence of said probe.

9. A composite probe, suitable for hybridizing to a target nucleotide sequence, comprising at least one probe, said probe comprising a polynucleotide sequence complementary to said target nucleotide sequence, said composite probe further comprising a polynucleotide ring unit, wherein said polynucleotide ring unit is joined to said probe to form an incipient closed loop.

10. The composite probe of Claim 9, comprising a first and second probe, wherein said first probe comprises a sequence complementary to a first region of said target nucleotide sequence, and wherein said second probe comprises a sequence complementary to a second region of said target sequence, wherein said ring unit is joined to said first probe and to said second probe, thereby forming an incipient closed loop.

reporters, wherein said second reporter produces a signal distinct produces a signal distinct from a signal produced by said first reporter;

(f) treating the probes and RING-LOCK Units, at this step or another step, to effect cross linking or to increase binding;

(g) hybridizing said first and second sense probes to the sense strand, thereby forming a sense hybridized complex; and hybridizing said first and second antisense probes to the antisense strand, thereby forming an antisense hybridized complex;

(h) ligating said first and second sense probes, thereby forming a closed loop or an incipient closed loop around the sense target strand; and ligating said first and second antisense probes; thereby forming a closed loop or an incipient closed loop around the antisense target strand;

(i) joining at least one first single-stranded polynucleotide to said first distal linker of said first sense probe, if not previously joined; joining at least one first single-stranded polynucleotide to said second distal linker of said second sense probe, if not previously joined; joining at least one second single stranded polynucleotide to said first distal linker of said first antisense probe, if not previously joined; joining at least one second polynucleotide to said second distal linker of said second antisense probe, if not previously joined;

(j) joining one or more first reporters to at least one said first single-stranded polynucleotide, if not so previously joined; joining one or more second reporters to at least one said second single-stranded polynucleotide, wherein said second reporter produces a signal distinct from said first reporter;

(k) determining whether the sense target sequence is present by detecting the presence or absence of said first reporter or reporters; or determining whether the antisense target sequence is present by detecting the presence or absence of said second reporter or reporters; or both.

19. The method of Claim 14, for use under liquid or near-liquid hybridization conditions, wherein said first probe comprises a distal end that is joined to a capture moiety, and said

stranded target nucleotide sequence and a nucleic acid probe, said WRAP-PROBE comprising a central sequence complementary to the single-stranded target nucleotide sequences, and further comprising a probe linker at one or both terminal ends, said probe linker comprising a single-stranded nucleotide sequence that does not hybridize to the target sequence; wherein said probe linker sequence is joined to one or more reporters, either prior to or subsequent to the hybridization of the probe to the target sequence;

c) washing to remove any unbound probe;

d) joining said reporter to said probe linker, if not previously joined;

e) detecting the presence or said reporter or reporters to indicate the target sequence.

29. The method of Claim 28, wherein the probe comprises a first terminal probe linker.

30. The method of Claim 28, wherein the probe comprises a first terminal probe linker and a second terminal probe linker.

31. The method of Claim 30, wherein the reporter is a labeled, double-stranded polynucleotide sequence, known as a GENE-TAG, having one or more said first reporter linkers, said first reporter linker comprising a single-stranded nucleotide sequence hybridized to the first terminal probe linker.

32. The method of Claim 30, wherein on more reporters comprise a reporter array, said array comprising a first labeled, double-stranded polynucleotide sequence, known as a GENE-TAG, linked together end-to-end by hybridization to one or more GENE-TAGS, wherein said first GENE-TAG comprises one or more first reporter linkers, said first reporter linker comprising a single-stranded nucleotide sequence hybridizable to said first terminal probe linker, and one or more second reporter linkers hybridized to one or more first reporter linkers of a second GENE-TAG, and wherein said second GENE-TAG further comprises one or more second reporter linkers, and optionally, additional GENE-TAGs linked to the remainder of the array by hybridization of the first reporter linker of each subsequent GENE-

TAG to the second reporter linker of the preceding GENE-TAG in the array, to form a chained or branch configuration having one or more ends, wherein said terminal end comprises the second reporter linker of a terminal GENE-TAG.

33. The method of Claim 32, further comprising one or more terminators, said terminator comprising a single-stranded polynucleotide sequence complementary to said second reporter linker of one or more terminal GENE-TAGs, such that said terminator forms said terminal end.

34. The method of Claim 28, wherein the reporter is joined directly to the first terminal probe linker.

35. The method of Claim 28, wherein the reporter is joined indirectly to the first terminal probe linker, further comprising a multi-linking unit interposed between said reporter and terminal probe linker, said unit known as a Multi-LINKER, comprising a first polynucleotide comprising a first terminal unit linker, a second terminal unit linker, and at least one internal linker, wherein said first terminal unit linker is hybridized to said first terminal probe linker, and wherein at least two of said internal and second terminal unit linkers are hybridized to one or more reporters, and wherein said first and second terminal unit linkers and said internal linker are not hybridizable to a target sequence of said probe.

36. The method Claim 35, further comprising one or more additional polynucleotides, said additional polynucleotide comprising a first terminal unit linker, a second terminal unit linker, at least one internal linker, wherein the first polynucleotide is joined to the additional polynucleotide by hybridization of the additional polynucleotide to the internal or second terminal linker of the first polynucleotide, and wherein said first and second terminal linkers and said internal linker are not hybridizable to a target sequence.

37. The method of Claim 30, wherein the reporter is a short oligonucleotide having a label unit.

38. A WRAP-LOCK method for providing circular enclosure of the target polynucleotide strand with a WRAP-PROBE, comprising:

a) providing the WRAP-PROBE of Claim 30;

b) providing a RING-LOCK Unit comprising at least two single-stranded polynucleotides, wherein at least one single-stranded polynucleotide is joined or suitable for joining to said first terminal probe linker, and wherein at least one single-stranded polynucleotide is joined or suitable for joining to one or more reporters;

c) treating the probe and RING-TAIL unit, at this step or another step, to effect cross linking or to increase binding;

c) hybridizing the WRAP-PROBE to the target strand, thereby forming a hybridized complex;

d) joining at least one single-stranded polynucleotide to said first terminal probe linker, if not previously joined,

e) providing a looping nucleotide comprising a first region complementary to said second terminal probe linker, and a second region complementary to said RING-TAIL Unit, wherein said looping nucleotide hybridizes to the second terminal probe linker and the RING-TAIL Unit, thereby forming a closed loop about the target strand;

f) joining a reporter or reporters to at least one ring-tail linker, if not previously attached, or if more or needed; and

g) detecting the presence of reporter to indicate the target sequence.

39. A DOUBLE WRAP-LOCK method of simultaneously detecting a target sequence on both a sense and anti-sense strand of DNA, comprising:

a) providing a double-stranded DNA comprising an antisense strand and a sense strand, wherein both said antisense strand and said sense strand comprise a target sequence;

b) providing a sense WRAP-PROBE of Claim 30, said sense WRAP-PROBE probe having a central sequence complementary to the sense strand target sequence;

c) providing an antisense WRAP-PROBE probe of Claim 30, said antisense WRAP-LOCK probe having a central sequence complementary to the antisense strand target sequence;

d) providing a first RING-TAIL Unit comprising at least two first single-stranded polynucleotides, wherein at least one first single-stranded polynucleotide is joined or suitable for joining to said first terminal probe linker of said sense WRAP-PROBE, and wherein at least one first single-stranded polynucleotide is joined or suitable for joining to one or more first reporters;

e) providing a second RING-LOCK Unit comprising at least two second single-stranded polynucleotides, wherein at least one second single-stranded polynucleotide is joined or suitable for joining to said first terminal probe linker of said antisense WRAP-PROBE, and wherein at least one second single-stranded polynucleotide is joined or suitable for joining to one or more second reporters, wherein said second reporter produces a signal distinct from said first reporter;

(f) treating the WRAP-PROBEs and the RING-LOCK Units, at this step of another step, to effect cross linking or to increase binding;

(g) hybridizing said sense WRAP-PROBE to the sense strand, thereby forming a sense hybridized complex; and hybridizing said antisense WRAP-PROBE to the antisense strand, thereby forming an antisense hybridized complex;

(h) joining at least one first single-stranded polynucleotide to said first terminal probe linker of said sense WRAP-PROBE, if not so previously joined; and joining at least one second single-stranded polynucleotide to said first terminal probe linker of said antisense WRAP-PROBE, if not so previously joined;

(i) providing a first looping nucleotide comprising a first region complementary to said second terminal probe linker of said sense WRAP-PROBE, and a second region complementary to said first RING-TAIL Unit, wherein said looping nucleotide hybridizes and thereby forms a closed loop about the target sense strand; and providing a second looping nucleotide comprising a first region complementary to said second terminal probe linker of said antisense WRAP-PROBE, and a second region complementary to said second RING-

TAIL Unit, wherein said looping nucleotide hybridizes and thereby forms a closed loop about the target antisense strand;

(j) joining one or more first reporters to at least one said first single-stranded polynucleotide, if not so previously joined; joining one or more second reporters to at least one said second single-stranded polynucleotide, if not previously joined, wherein said second reporter produces a signal distinct from said first reporter;

(k) determining whether the sense target sequence is present by detecting the presence or absence of said first reporter or reporters; or determining whether the antisense target sequence is present by detecting the presence or absence of said second reporter or reporters, or both.

40. A GENE-TAG method for exponential signal amplification for the detection of a target nucleotide sequence, comprising:

a) providing a single-stranded target nucleic acid sequence;

b) providing a probe comprising a sequence hybridizable to said target nucleic acid sequence, and a terminal linker sequence on one or both ends of the probe, wherein said linker sequence is a single-stranded polynucleotide hybridizable to a complementary sequence;

c) providing one or more GENE-TAGs, said GENE-TAG comprising a labeled, double-stranded polynucleotide reporter having one or more first reporter linkers and one or more second reporter linkers opposed to said first reporter linker, said reporter linkers comprising single-stranded polynucleotide sequences not hybridizable to the target sequence, wherein at least one said GENE-TAG has a first reporter linker complementary to said probe terminal linker, and wherein one or more said reporter linkers link together end-to-end by hybridization of the first reporter linker of each subsequent GENE-TAG to the second reporter linker of each preceeding GENE-TAG in the array, to form a chained or branched configuration comprising one or more terminal end, wherein said terminal end comprises the second reporter linker of a terminal

GENE-TAG; wherein said GENE-TAG is joined to said probe and this or a subsequent step;

d) hybridizing said probe to said target nucleotide sequence;

e) joining said GENE-TAG or GENE-TAGs to said probe, if not so previously joined;

f) determining the presence of the target sequence by detecting the presence of the GENE-TAG or GENE-TAGs. .

41. The method of Claim 40, wherein the first and second reporter linkers of the GENE-TAGs are complementary, such that only GENE-TAGs of one type are required to form chained or branched reporter arrays.

42. The method of Claim 41, further comprising one or more terminators, said terminator comprising a single-stranded polynucleotide sequence complementary to said second reporter linker of said terminal GENE-TAG, such that said terminator forms said terminal end.

43. The method of Claim 42, wherein the length of said chained or branched reporter arrays, and thus probe amplification, is controlled by the ratio of GENE-TAGs to said terminators. .

44. The method of Claim 41, further comprising a Terminator TAG, said Terminator TAG comprising a GENE-TAG having no second reporter linker, wherein said first reporter linker of said Terminator TAG hybridizes to the second reporter linker of a terminal GENE-TAG of the chain or branch.

45. The method of Claim 44, wherein the length of said chained or branched reporter arrays, and thus probe amplification, is controlled by the ratio of GENE-TAGs to Terminator TAG.

46. The method of Claim 40, wherein only one GENE-TAG is provided, said GENE-TAG having no second reporter linker.

47. The method of Claim 40, wherein said first and second linkers of said GENE-TAGs are not complementary, such that at least two types of GENE-TAGs are required to form chained or branched arrays, wherein GENE-TAGs of a first type comprise a first reporter linker complementary to the probe terminal linker, and a second reporter linker complementary to the first reporter linker of GENE-TAG of the second type, and wherein said GENE-TAG of the second type has a first reporter linker complementary to the second reporter linker of a GENE-TAG of the first type, such that only a GENE-TAG of the first type will bind to a GENE-TAG of the second type, and only a GENE-TAG of the second type will bind to a GENE-TAG of the first type; wherein said chained or branched arrays are formed by providing GENE-TAGs sequentially, alternating between the first and second type, and thereby forming alternating layers of GENE-TAGs of a first or second type upon the probe.

48. The method of Claim 40, wherein said GENE-TAGS are constructed by PCR amplification of arbitrary template DNA using one or more modified oligonucleoties as primers, wherein each modified oligonucleotide has one or more internal spacers, whereby the first and second terminal single-stranded linkers are preserved.

49. The method of Claim 40, wherein said GENE-TAGs are constructed by hybridization of two polynucleotides, said first polynucleotide having a second terminal end complementary to the first terminal end of a second polynucleotide.

50. The method of Claim 49, wherein said first polynucleotide has an internal sequence complementary to the internal sequence of a second polynucleotide, forming a GENE-TAG having side arms, said side arms comprising the second terminal end of the first polynucleotide, and the first terminal end of the second polynucleotide, said GENE-TAGs known as TINKER-TAGS.

51. The method of Claim 40, wherein the GENE-TAG is labeled directly.

52. The method of Claim 40, wherein the GENE-TAG is labeled indirectly.

53. The method of Claim 50, wherein said TINKER-TAGs are labeled indirectly by hybridization of said side arms to short oligonucleotides conjugated to one or more labeling agents.

54. The method of Claim 53, wherein said TINKER-TAGs are labeled indirectly by hybridization of said side arms to short oligonucleotides conjugated to gold particles, said gold particles coated in silver to form dense clusters.

55. A method of constructing probes detectable on the basis of a unique signal, comprising:

a) providing a probe hybridizable to a single-stranded nucleotide sequence, wherein said probe comprises a terminal linker at one or both ends, said terminal linker comprising a single-stranded polynucleotide capable of binding to a complementary sequence;

b) preparing on or more COLOR-TAGs, said COLOR-TAG comprising a double-stranded polynucleotide reporter having a first reporter linker and a second reporter linker opposed to said first reporter linker, wherein said COLOR-TAG is labeled with one or more labels or colors, or both, and wherein said first and second reporter linkers are specific to the labels or colors employed; wherein at least one COLOR-TAG terminal linker is hybridizable to the terminal linker of said probe;

c) hybridizing one or more said COLOR-TAGs to the probe, at this step or a later step;

e) hybridizing said probe to said target sequence;

f) hybridizing said COLOR-TAG to the probe, if not so previously hybridized;

f) determining the presence of said target sequence by detecting the presence of said COLOR-TAG.

56. The method of Claim 55, wherein said Color-TAG is labeled with two or more different labels or colors.

57. The method of Claim 55, further comprising a multilinking unit suitable for joining said probe to one or more COLOR-TAGs, said multilinking unit comprising a proximal linker for joining said multilinking unit to said probe terminal linker, and one or more distal linkers known as COLOR-LINKERS, said COLOR-LINKERS complementary to one or more of said COLOR-TAG terminal linkers, wherein a specific mic of COLOR-TAGS is captured by said multilinking unit depending on the specific ratio of COLOR-LINKERS used to prepare the multilinking unit, wherein said multilinking unit is hybridized to said COLOR-TAGS, thereby forming a COLOR-TAG complex, either prior or subsequent to the hybridization of the multilinking unit to the probe, and wherein said multilinkining unit is hybridized to said probe either prior to or subsequent to the hybridization of the probe to said target sequence.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/16242

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12Q 1/68; C12P 19/34; C07H 21/02, 21/04

US CL : 436/6, 91.1, 91.2; 536/22.1 24.3 24.31 24.32, 24.33

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 436/6, 91.1, 91.2; 536/22.1 24.3 24.31 24.32, 24.33

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,695,935 A (MANDRAND et al) 09 December 1997, see entire document.	1-57
Y	US 5,624,802 A (URDEA et al) 29 April 1997, see entire document.	1-57
Y	US 5,681,697 A (URDEA et al.) 28 October 1997, see entire document.	1-57
Y	US 5,571,677 A (GRYAZNOV) 05 November 1996, see entire document.	1-57
Y,P	US 5,853,993 A (DELLINGER et al) 29 December 1998, see entire document	1-57
Y,P	US 5,912,124 A (KUMAR) 15 June 1999, see entire document.	1-57

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

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Date of the actual completion of the international search

18 OCTOBER 1999

Date of mailing of the international search report

21 OCT 1999

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Form PCT/ISA/210 (second sheet)(July 1992)☆

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/16242

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

WEST 1.2, CAPLUS, MEDLINE, BIOSIS, EMBASE BIOTECHDS.
oligo, nucleic, DNA, RNA, dendrimer, multimer, polymer, oligomer, signal, amplification, padlock, circular, probe, primer, lock, hybridize, anneal

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REVISED VERSION

INTERNATIONAL SEARCH REPORT

Internati nal applicati n No.
PCT/US99/16242

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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

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Date of the actual completion of the internati nal search
18 OCTOBER 1999

Date f mailing of the international search report
1 1 MAY 2000

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International application N .
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C (Continuati n). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citati n f document, with indicati n, where appropriate, f the relevant passages	Relevant to claim N .
Y	WO 92/17484 A1 (RESEARCH CORPORATION TECHNOLOGIES, INC.) 15 October 1992, see entire document.	1-57
Y	NILSSON et al. Padlock probes: circularizing oligonucleotides for localized DNA detection. Science. 30 September 1994, Vol. 265, pages 2085-2088, see entire document.	1-57
Y	Database CAPLUS on STN, VOGELBACKER et al. DNA dendrimers: assembly and signal amplification. Book of Abstracts, 213th ACS National Meeting, San Francisco. April 1997, abstract only.	1-57

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